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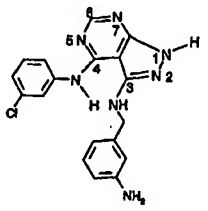
(54) Title: FUSED PYRAZOLE DERIVATIVE AND PROCESS FOR ITS PREPARATION

(57) Abstract

3–(3–Aminobenzylamino)–4–(3–chlorophenylamino)–1H–pyrazolo[3,4–d]pyrimidine of formula (I) is described. The compound of formula (I) inhibits, in particular, tyrosine–kinase activity of the receptor for the epidermal growth factor and can be used, for example, as antitumour agent and in cases of epidermal hyperproliferation (psoriasis).



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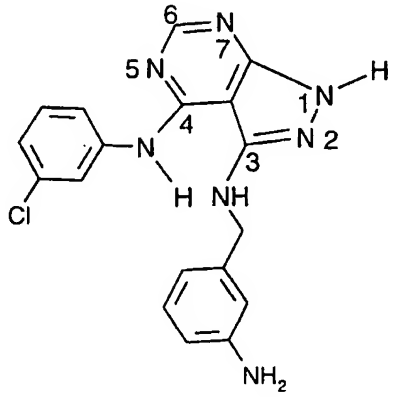
Fused pyrazole derivative and process for its preparation

The invention relates to 3-(3-aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine, its salts, solvates and tautomers, and to a process for its preparation, pharmaceutical formulations which comprise 3-(3-aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine or its pharmaceutically acceptable salts or solvates, and the use of these derivatives as pharmaceuticals.

3-(3-Aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine has formula I.



(I)

The general terms used hereinafter preferably have the following meanings for the purpose of the present application:

The prefix "lower" designates a radical having up to a maximum of 7, in particular having up to a maximum of 4, and especially having 1 or 2, carbon atoms.

Salts of the compound of the formula I are, because it has basic properties, acid addition salts with organic or inorganic acids, in particular the pharmaceutically acceptable non-toxic salts. Examples of suitable inorganic acids are carbonic acid (preferably in the form of carbonates or bicarbonates); hydrohalic acids such as hydrochloric acids, sulfuric acid; or phosphoric acid. Examples of suitable organic acids are carboxylic, phosphonic, sulfonic or sulfamic acids, for example acetic acid, propionic acid, octanoic acid, decanoic acid,

dodecanoic acid, glycolic acid, lactic acid, 2-hydroxybutyric acid, gluconic acid, glucosemonocarboxylic acid, fumaric acid, succinic acid, adipic acid, pimelic acid, suberic acid, azelaic acid, malic acid, tartaric acid, citric acid, glucaric acid, galactaric acid, amino acids such as glutarmic acid, aspartic acid, N-methylglycine, acetylaminoacetic acid, N-acetylasparagine or N-acetylcystein, pyruvic acid, acetoacetic acid, phosphoserine, 2- or 3-glycerophosphoric acid, glucose-6-phosphoric acid, glucose-1-phosphoric acid, fructose-1,6-bisphosphoric acid, maleic acid, hydroxymaleic acid, methylmaleic acid, cyclohexane-carboxylic acid, adamantanecarboxylic acid, benzoic acid, salicylic acid, 1- or 3-hydroxy-naphthalene-2-carboxylic acid, 3,4,5-trimethoxybenzoic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, 4-aminosalicylic acid, phthalic acid, phenylacetic acid, mandelic acid, cinnamic acid, nicotinic acid, isonicotinic acid, glucuronic acid, galacturonic acid, methane- or ethanesulfonic acid, 1-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, benzenesulfonic acid, 1-naphthalenesulfonic acid, 1,5-naphthalenedisulfonic acid, 2-, 3- or 4-methylbenzenesulfonic acid, methylsulfuric acid, ethylsulfuric acid, dodecylsulfuric acid, N-cyclohexylsulfamic acid, N-methyl-, N-ethyl- or N-propylsulfamic acid or other organic protic acids such as ascorbic acid.

It is also possible to use pharmaceutically unsuitable salts such as pikrates or perchlorates for isolation or purification. Only the pharmaceutically acceptable (in appropriate doses), non-toxic salts are used therapeutically and are therefore preferred.

The term "solvates" also embraces in particular hydrates.

The compound of the formula I and intermediates which comprise a pyrazole residue for preparing it may, under certain conditions, for example when they are dissolved in certain solvents, be partly in a tautomeric form in which the hydrogen atom which is normally located on nitrogen N-1 has shifted to another suitable nitrogen atom, for example N-2, N-5 or N-7. The invention also relates to these tautomers.

The compound of the formula I and its pharmaceutically acceptable salts, solvates and tautomers have valuable pharmacological properties. In particular, they show specific inhibitory effects which are of pharmacological interest. They act primarily as protein tyrosine kinase inhibitors. For example, they show a potent inhibition of the tyrosine kinase activity of the receptor for epidermal growth factor (EGF) and of c-erbB2 kinase. These

receptor-specific enzyme activities play a key role in signal transmission in a large number of mammalian cells, including human cells, in particular of epithelial cells, cells of the immune system and cells of the central and peripheral nervous systems. For example, the EGF-induced activation of the receptor-associated protein tyrosine kinase (EGF-R-PTK) is in various types of cells a prerequisite for cell division and thus proliferation of the cell population. Multiplication of EGF receptor-specific tyrosine kinase inhibitors thus inhibits multiplication of the cells. Analogous statements apply to the other protein kinases mentioned hereinbefore and hereinafter.

The inhibition of the EGF receptor-specific protein tyrosine kinase (EGF-R-PTK) can be detected by known methods, for example using the recombinant intracellular domain of the EGF receptor (EGF-R ICD; see, for example, E. McGlynn et al., Europ. J. Biochem. 207, 265-275 (1992)). The compound of the formula I and its salts, solvates and tautomers inhibit the enzyme activity compared with the control without inhibitor by 50% (IC50) for example in a concentration of 0.001 to 0.003 µM.

The compound of the formula I and its salts and solvates likewise show in the micromolar range for example also an inhibition of cell growth in EGF-dependent cell lines, for example the epidermoid BALB/c mouse keratinocyte cell line (see Weissmann, B.A., and Aaronson, S.A., Cell 32, 599 (1983)) or the A431 cell line, which are acknowledged to be useful standard sources of EGF-dependent epithelial cells (see Carpenter, G., and Zendegni, J. Anal. Biochem. 153, 279-282 (1985)). The inhibitory effect of the compounds of the formula I is measured in a known test method (see Meyer et al., Int. J. Cancer 43, 851 (1989)) briefly as follows: BALB/MK cells (10,000/microtitre plate well) are transferred into 96-well microtitre plates. The test compounds (dissolved in DMSO) are added in a series of concentrations (dilution series) so that the final concentration of DMSO is not greater than 1% (v/v). After the addition, the plates are incubated for three days, during which the control cultures without test substance are able to pass through at least three cell division cycles. The growth of the MK cells is measured by means of methylene blue staining: after the incubation, the cells are fixed with glutaraldehyde, washed with water and stained with 0.05% methylene blue. After a washing step, the dye is eluted with 3% HCl, and the optical density of each well of the microtitre plate is measured using a Titertek multiskan at 665 nm. IC_{50} -values are determined by a computer-assisted system using the formula:

$IC_{50} = [(OD_{Test} - OD_{Start})/(OD_{Control} - OD_{Start})] \times 100.$

The IC_{50} value in these experiments is reported as the concentration of the particular test compound which results in a cell count which is 50% less than the control without inhibitor. The compound of the formula I and its pharmaceutically acceptable salts show inhibitory effects in the micromolar region, for example an IC_{50} of about 0.1 μM.

The compound of the formula I and its pharmaceutically acceptable salts and solvates also show *in vivo* an inhibition of the growth of tumour cells, for example as shown by the test described hereinafter: The test is based on the inhibition of the growth of the human epidermoid carcinoma A431 (ATCC No. CRL 1555; American Type Culture Collection, Rockville, Maryland, USA; see Santon, J.B., et al., Cancer Research 46, 4701-4705 (1986) and Ozawa, S., et al., Int. J. Cancer 40, 706-710 (1987)), which is transplanted into female BALB/c nude mice (Bomholtgard, Denmark). This carcinoma shows a growth which correlates with the extent of EGF receptor expression. In experiment, tumours which have been grown *in vivo* and have a volume of about 1 cm^3 are removed surgically from experimental animals under sterile conditions. These tumours are comminuted and suspended in 10 volumes (w/v) of phosphate-buffered saline. The suspension is injected s.c. (0.2 ml/mouse in phosphate-buffered saline) into the left flank of the animals. Alternatively, 1 x 10^6 cells from an *in-vitro* culture in 0.2 ml of phosphate-buffered saline can be injected. Treatment with the test compound of the formula I or a salt thereof is started 5 or 7 days after the transplantation when the tumours have reached a diameter of 4-5 mm. The particular active substance is administered (in various doses in different animal groups) once a day for 15 consecutive days. The tumour growth is determined by measuring the diameters of the tumours along three mutually perpendicular axes. The tumour volumes are calculated using the known formula p x L x $D^2/6$ (see Evans, B.D., et al., Brit. J. Cancer 45, 466-8 (1982)). The results are reported as treated/control percentages (T/C x 100 = T/C%). With a dose of 3 to 50 mg/kg of the active substance, marked inhibition of tumour growth is found, for example T/C% values of less than 10, which denotes strong inhibition of tumour growth.

One advantage of the compound of the formula I and its pharmaceutically acceptable salts is that they have outstanding efficacy associated with excellent selectivity. Thus, for example, the protein tyrosine kinases involved in signal transmission mediated by tropic factors, for example the abl kinases, such as, in particular v-abl kinase, kinases from the family of src kinases, such as, in particular, c-src kinase, and the serine/threonine kinases, for example the protein kinase C, and cdc2 kinase, all of which play a part in growth regulation and transformation of mammalian cells, including human cells, are inhibited only much more weakly or virtually not at all. This also applies to enzymes such as Flt1, Flk, c-Met and Tek. As can be easily understood, this is presumably also associated with fewer side effects. Another advantage of these compounds is the high cell permeability and the high blood levels which are reached.

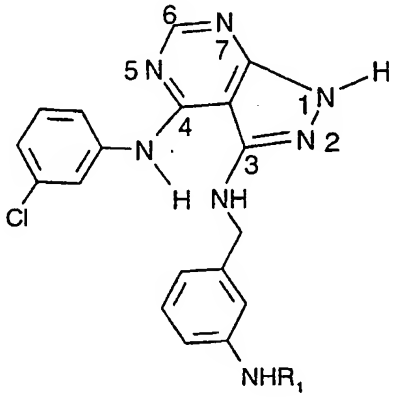
The compound of the formula I and its pharmaceutically acceptable salts which inhibit the tyrosine kinase activity of the receptor for epidermal growth factor (EGF) can therefore be used, for example, for treating benign or malignant tumours, for example those of the lung, of the breast, of the bladder, of the skin or of the intestine. They are able to bring about tumour regression and to prevent tumour metastasis and the growth of micrometastases. They can be used in particular in cases of epidermal hyperproliferation (psoriasis), in the treatment of neoplasms of an epithelial nature, for example carcinomas of the breast, and for leukaemias. The compound of the formula I and its pharmaceutically acceptable salts can furthermore be employed for treating disorders of the immune system involving protein tyrosine kinases which are inhibited; this compound of the formula I can also be used to treat disorders of the central or peripheral nervous system as long as signal transmission by these protein tyrosine kinases is involved.

The present invention generally also relates to the use of the compound of the formula I and its pharmaceutically acceptable salts for inhibiting said protein kinases.

The compound of the formula I according to the invention and its pharmaceutically acceptable salts, solvates or tautomers can be used either alone or else in combination with other pharmacologically effective substances, for example together with inhibitors of the enzymes of polyamine synthesis, inhibitors of protein kinase C, inhibitors of other tyrosine kinases, cytokines, negative growth regulators, for example TGF-ß or IFN-ß, aromatase inhibitors, antioestrogens and/or cytostatics.

The compound of the formula I and its salts can be prepared in a manner known per se, for example as exemplified in the example part. The preparation process according to the invention comprises

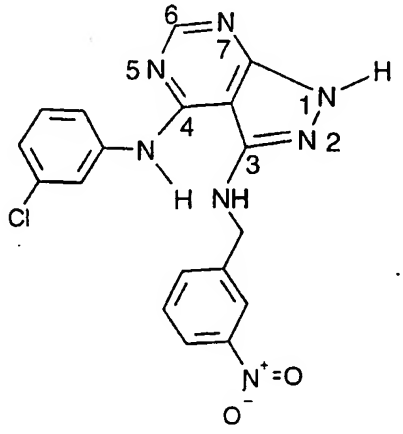
a) eliminating the protective group from a compound of the formula II



(II)

in which R_1 is an aminoprotective group, or

b) converting the nitro group in the compound of the formula III



(III)

into an amino group using a suitable reducing agent,

and if required converting a compound of the formula I obtained by process a) or b) into a salt, or converting a resulting salt of a compound of the formula I into the free compound.

The procedure for these process variants and the preparation of the starting materials are described in detail hereinafter:

Process a): Suitable aminoprotective groups such as, in particular, tert-butyloxycarbonyl (Boc), and their elimination, are familiar to the skilled person. Thus, for example, the Boc protective group can be eliminated in an acidic medium, for example by adding 3-normal methanolic hydrochloric acid.

Process b): A suitable reducing agent is, in particular, hydrogen in the presence of a suitable catalyst such as, in particular, Raney nickel.

Acid addition salts of the compound of the formula I are obtained in a manner known per se, for example by treatment with an acid or a suitable anion exchanger.

Acid addition salts can be converted in a conventional way into the free compound, for example by treatment with a suitable basic agent.

Solvates often form "automatically" during working up of the reaction mixture, and hydrates form on standing in air.

The preparation of the starting materials of the formulae II and III is described in the example part.

Mixtures of isomers can be resolved in a manner known per se, for example by fractional crystallization, chromatography etc., into the individual isomers.

The processes described above, including the processes for eliminating protective groups and the additional process measures, are, unless indicated otherwise, carried out in a manner known per se, for example in the presence or absence of, preferably inert, solvents and diluents, if necessary in the presence of condensing agents or catalysts, at reduced or elevated temperature, for example in a temperature range from about -20°C to about 150°C, in particular from about 0°C to about +70°C, preferably from about +10°C to about

+50°C, mainly at room temperature, in a suitable vessel, and, if necessary, in an inert gas, for example nitrogen, atmosphere.

Moreover, taking account of all the substituents present in the molecule, if necessary, for example in the presence of easily hydrolysable radicals, particularly mild reaction conditions should be used, such as short reaction times, use of mild acidic or basic agents in low concentration, stoichiometric ratios of amounts, choice of suitable catalysts, solvents, temperature and/or pressure conditions.

The invention also relates to those embodiments of the process which start from a compound which can be obtained as intermediate at any stage of the process, and the missing process steps are carried out, or the process is stopped at any stage, or a starting material forms under the reaction conditions or is used in the form of a reactive derivative or salt. The starting materials preferably used are those which in the process result in the compounds described above as particularly valuable.

Because of the close relationship between the free compounds of the formulae I, II and III, and other intermediates and their salts, solvates, such as, in particular, hydrates, and tautomers, mention only of the free compound in this text also means, where logical and expedient, a salt, solvate or tautomer.

The invention also relates to a method for the treatment of warm-blooded animals suffering from an oncosis, wherein an effective tumour-inhibiting amount of a compound of the formula I or of a pharmaceutically acceptable salt or solvate thereof is administered to warm-blooded animals requiring such a treatment. The invention additionally relates to the use of a compound of the formula I or of a pharmaceutically acceptable salt or solvate thereof for inhibiting EGF receptor-specific protein tyrosine kinase C in warm-blooded animals or for manufacturing pharmaceutical products for use for the therapeutic treatment of the human or animal body. This entails administering to a warm-blooded animal with a body weight of about 70 kg an effective dose depending on the species, age, individual condition, mode of administration and the particular pathology, for example daily doses of about 5-5000 mg, in particular 200-2000 mg.

The invention also relates to pharmaceutical products which comprise an effective amount, in particular an amount effective for the prophylaxis or therapy of one of the above-mentioned diseases, of the active substance together with pharmaceutically acceptable carriers which are suitable for topical, enteral, for example oral or rectal, or parenteral administration, and may be inorganic or organic, solid or liquid. Used for oral administration are, in particular, tablets or gelatin capsules which comprise the active ingredient together with diluents, for example lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycerol, and/or lubricants, for example diatomaceous earth, talc, stearic acid or salts thereof, such as magnesium or calcium stearate, and/or polyethylene glycol. Tablets may likewise comprise binders, for example magnesium aluminium silicate, starches, such as maize, wheat or rice starch, gelatin, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, and, if required, disintegrants, for example starches, agar, alginic acid or a salt thereof, such as sodium alginate, and/or effervescent mixtures, or adsorbents, colorants, flavourings and sweeteners. The pharmacologically active compounds of the present invention can furthermore be used in the form of products which can be administered parenterally or of infusion solutions. Solutions of this type are preferably isotonic aqueous solutions or suspensions, it being possible for the latter, for example in the case of lyophilized products which comprise the active substance alone or together with a carrier, for example manitol, to be prepared before use. The pharmaceutical products may be sterilized and/or comprise adjuncts, for example preservatives, stabilizers, wetting agents and/or emulsifiers, solubilizers, salts to regulate the osmotic pressure and/or buffers. The present pharmaceutical products which, if required, may comprise further pharmacologically active substances, such as antibiotics, are manufactured in a manner known per se, for example using conventional mixing, granulating, coating, dissolving or lyophilizing processes, and comprise about 1% to 100%, in particular about 5% to about 90%, of the active substance(s).

The following examples illustrate the invention without restricting it in any way. The ratio of the solvents or eluents to one another in the solvent or eluent mixtures used is stated in proportions by volume (V/V), and temperatures are stated in degrees Celsius.

Abbreviations:

abs.: absolute

Boc: tert-butyloxycarbonyl
DMEU: 1,3-dimethyl-2-imidazolidinone
DMF: dimethylformamide
ESI-MS: Electrospray Ionisation Mass Spectroscopy
sat.: saturated
h: hour(s)
HV: high vacuum
conc.: concentrated
min: minute(s)
RF: reflux
RT: room temperature
RV: rotary evaporator
brine: saturated sodium chloride solution
THF: tetrahydrofuran

Example 1: 3-(3-Aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine
A mixture of 0.15 g (0.379 mmol) of 4-(3-chlorophenylamino)-3-(3-nitrobenzylamino)-1H-pyrazolo[3,4-d]pyrimidine, 4 ml of methanol, 4 ml of THF and 40 mg of Raney nickel is hydrogenated at RT under atmospheric pressure until hydrogen uptake ceases. The mixture is filtered, and the filtrate is concentrated in vacuo, and the residue is recrystallized from ethyl acetate/hexane, resulting in the title compound with a water content of 1.22%; melting point 165-170°C; ESI-MS: $(M+H)^+ = 366$.

The starting material is obtained in the following way:

Stage 1.1: 43.6 ml (400 mmol) of benzylamine are added to a suspension of 68.4 g (400 mmol) of 3,3-bis(methylthio)-2-cyanoacrylonitrile [3,3-bis(methylsulfanyl)-2-cyano-acrylonitrile; Maybridge] in 400 ml of ethyl acetate. The clear solution is slowly heated to 70 °C (→ MeSH evolution), stirred at this temperature for 1.5 h, cooled to RT and evaporated, resulting in crystalline 3-benzylamino-3-methylthio-2-cyanoacrylonitrile; ^{1}H-NMR: (CD_3OD) 7.36 (m, 5H), 4.77 (s, 2H), 2.59 (s, 3H).

Stage 1.2: 24 ml (0.48 mol) of hydrazine hydrate are added dropwise to a solution of 92 g (0.4 mol) of 3-benzylamino-3-methylthio-2-cyanoacrylonitrile in 400 ml of methanol. During

this, the temperature rises to 40°C. It is slowly heated to boiling (→ MeSH evolution), boiled for 2 h, cooled to RT and evaporated to a residual volume of 200 ml. Dilution with diethyl ether, filtration and washing with diethyl ether afford 5-amino-3-benzylamino-1H-pyrazole-4-carbonitrile [*Spectrochimica Acta*, 47A, 1635 (1991)]; melting point 150-152°C; TLC: R_f = 0.41 (ethyl acetate).

Stage 1.3: A suspension of 74.3 g (348 mmol) of 5-amino-3-benzylamino-1H-pyrazole-4-carbonitrile in 1.0 litre of toluene is boiled with 70.1 ml (95% pure, 409 mmol) of N,N-dimethylformamide diethyl acetal under reflux under an N_2 atmosphere for 2 h. Cooling to RT, filtration with suction and washing with diethyl ether afford N'-(3-benzylamino-4-cyano-1H-pyrazol-5-yl)-N,N-dimethylformamidine; melting point 197-200°C; TLC: R_f = 0.50 (ethyl acetate).

Stage 1.4: 60 g (0.47 mol) of 3-chloroaniline are dissolved in 255 ml (0.56 mol) of 2.2 N methanolic HCl. Concentration and stirring of the residue in diethyl ether afford 3-chloroaniline hydrochloride after filtration and drying.

Stage 1.5: 79.2 g (295 mmol) of N'-(3-benzylamino-4-cyano-1H-pyrazol-5-yl)-N,N-dimethyl-formamidine are suspended in 700 ml of methanol with exclusion of moisture, 60.6 g (369 mmol) of 3-chloroaniline hydrochloride are added, and the mixture is boiled under reflux for 22 h. The resulting yellow reaction solution is cooled to 50°C and poured into 2 litres of ice-water, 200 ml of sat. $NaHCO_3$ solution and 1 l of ethyl acetate. The aqueous phase is separated off and extracted twice with ethyl acetate. The organic phases are washed twice with water, sat. $NaHCO_3$ solution, water and brine, dried (Na_2SO_4) and evaporated to a residual volume of ≈1.5 litre. Seeding and dilution with 300 ml of diethyl ether afford crystalline 3-benzylamino-4- (3-chlorophenylamino)-1H-pyrazolo[3,4-d]-pyrimidine; melting point 214-217 °C; TLC: R_f = 0.29 (ethyl acetate:hexane = 1:1).

Stage 1.6: Residual water is removed from a suspension of 75.8 g (216 mmol) of 3-benzylamino-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine in 1.5 litre of benzene by distilling off a little solvent. This suspension is then added, with exclusion of moisture, to 84 g of aluminium chloride (Fluka, Buchs/Switzerland) in 500 ml of benzene, and the mixture is heated at 80°C for 2.5 h. The reaction mixture is cooled to RT, the supernatant

benzene phase is poured into 2 kg of ice-water (a green oily residue remains behind), and the solid which separates out is filtered off with suction and thoroughly washed with water ($\rightarrow K_1$). The benzene is evaporated off from the filtrate in a rotary evaporator, the remaining aqueous phase is added together with 1 kg of ice to the green oily residue, and the mixture is hydrolysed at 40°C for 2 h. The crystalline product is filtered off with suction and washed with water ($\rightarrow K_2$). K_1 and K_2 are taken up in 1 litre of methanol, acidified with 4N aqueous HCl and partly evaporated. Water is added, and the methanol is completely evaporated off. The crystals are filtered off and washed with water. The same purification procedure is repeated with half-saturated Na_2CO_3 solution/methanol and water/methanol. Stirring in methanol at 50°C, precipitation with diethyl ether, filtration and drying afford 3-amino-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine; melting point 232-234°C;.TLC: R_f = 0.50 (ethyl acetate).

Stage 1.7: 4-(3-Chlorophenylamino)-3-(3-nitrobenzylamino)-1H-pyrazolo[3,4-d]pyrimidine
0.907 g (6 mmol) of 3-nitrobenzaldehyde is added to a solution of 1.043 g (4 mmol) of 3-amino-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine [see Stage 1.6] in 100 ml of methanol, 100 ml of DMEU and 0.48 g (8 mmol) of acetic acid, and the mixture is stirred at RT for 1 h. Then 2.07 g (28 mmol) of sodium cyanoborohydride (85%) are added to the reaction mixture, which is stirred at 20°C for 7 days. After addition of a further 2.07 g (28 mmol) of sodium cyanoborohydride (85%), the reaction mixture is stirred at RT for a further 7 days and then poured into 3.2 litres of water. Stirring overnight, filtration and recrystallization of the residue on the filter from ethanol afford the title compound; melting point 195-196°C; ESI-MS: $(M+H)^+$ = 396.

Example 2: 3-(3-Aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine
As an alternative to Example 1, the title compound is obtained in the following way:

A mixture of 125 g (0.2827 mol) of 3-[3-(N-Boc-amino)benzylamino]-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine and 2000 ml of 3N methanolic hydrochloric acid is stirred at RT for 3 h. Then 500 ml of diethyl ether are added to the reaction mixture, which is cooled to 5°C and filtered, and the residue on the filter is washed with 100 ml of methanol/diethylether (1:1) and 250 ml of diethyl ether. The filtered material is dried at 100°C under HV and then suspended in 2500 ml of water, 270 ml of saturated

aqueous sodium carbonate solution are added to the suspension, and the reaction mixture is stirred at RT for 1 h. Filtration, washing of the residue on the filter with about 1000 ml of cold water and drying (about 100 mbar, 15 h, 80°C and HV, 24 h, 100°C) afford the title compound with a water content of 2.31%; melting point 179-180°C; ESI-MS: $(M+H)^+$=366. (Yield: 90.4 %)

The starting material is prepared as follows:

Stage 2.1: 3-(N-Boc-amino)-benzylamine

50 g of Raney nickel are added to a solution of 182.5 g (0.8362 mol) of 3-(N-Boc-amino)-benzonitrile (for preparation, see: WO 93/19063) in 1500 ml of ethanolic ammonia solution (about 8% of NH_3). Hydrogenation is carried out under a pressure of 4 bar for 10 h and, after filtration, the filtrate is evaporated in vacuo. The crystalline residue is recrystallized from a mixture of 300 ml of ethyl acetate and 400 ml of hexane. Filtration, washing of the filtered material with hexane and drying in vacuo result in the title compound; melting point 133-134°C; ESI-MS: $(M+H)^+$=223. Evaporation of the mother liquor and recrystallization of the residue from ethyl acetate/hexane afford a 2nd batch of title compound; melting point 132-133°C.(Yield: 96.6 %)

Stage 2.2: 3-[3-(N-Boc-amino)benzylamino]-2-cyano-3-methylthioacrylonitrile

A mixture of 179 g (0.8053 mol) of 3-(N-Boc-amino)benzylamine and 137.1 g of 3,3-bismethylthio-2-cyanoacrylonitrile [for preparation, see: Chem. Ber. **95**, 2861 (1962)] and 1000 ml of ethyl acetate is heated under reflux for 2 h. Then 1000 ml of hexane are added to the reaction mixture. The crystals obtained on cooling to 0°C are filtered off, and the residue on the filter is washed with hexane. Drying in vacuo (about 100 mbar, 4 h, 80°C) results in the title compound; melting point 145-146°C; ESI-MS: $(M+H)^+$=345. (Yield: 96.4%)

Stage 2.3: 5-Amino-3-[3-(N-Boc-amino)benzylamino]-4-cyanopyrazole

A mixture of 267.5 g (0.7766 mol) of 3-[3-(N-Boc-amino)benzylamino]-2-cyano-3-methyl-thioacrylonitrile, 40.41 ml (0.8153 mol) of hydrazine hydrate and 800 ml of methanol is heated under reflux for 4 h and then evaporated in vacuo. Crystallization of the residue from about 1400 ml of ethyl acetate/hexane (1:1), filtration at 0°C, washing of the filtered material with hexane and drying under HV (8 h, 90°C) result in the title compound containing 8.2% ethyl acetate; melting point (decomposition) 113-114°C; ESI-MS: $(M+H)^+$ = 329. (Yield: 93.5%)

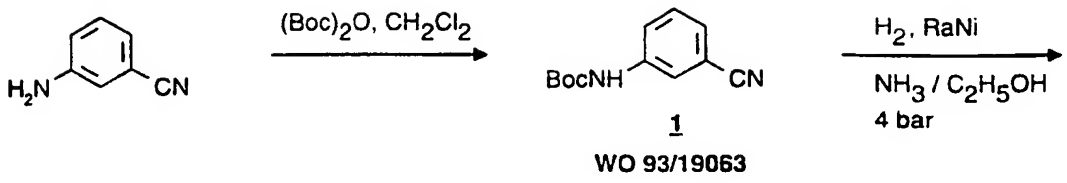
Stage 2.4: 3-[3-(N-Boc-amino)benzylamino]-4-cyano-5-dimethylaminomethyleneamino-pyrazole

A mixture of 246.6 g (0.6893 mol) of 5-amino-3-[3-(N-Boc-amino)benzylamino]-4-cyano-pyrazole, 177 ml (1.0018 mol) of N,N-dimethylformamide diethyl acetal (97%), 1000 ml of toluene and 200 ml of ethanol is heated under reflux for 2 h. Then about 1 l of hexane is added to the hot reaction mixture, which is then cooled in an ice bath. The crystalline precipitate which forms is filtered off and washed with 300 ml of hexane. Drying in vacuo under about 100 mbar (14 h, 90°C) results in the title compound containing 2.1% toluene; melting point 112-115°C; ESI-MS: $(M+H)^+$=384. (Yield: 90.1%)

Stage 2.5: 3-[3-(N-Boc-amino)benzylamino]-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine

A mixture of 242.4 g (0.6189 mol) of 3-[3-(N-Boc-amino)benzylamino]-4-cyano-5-dimethyl-aminomethyleneaminopyrazole (97.9%), 120 g (0.7316 mol) of 3-chloroaniline hydrochloride (for preparation, see Justus Liebigs Ann. Chem. **176**, 45 (1875) and 1000 ml of methanol is heated under reflux for 38 h. The resulting suspension is cooled to 20°C and then 100 ml of water are slowly added and, after filtration, the residue on the filter is washed with 150 ml of methanol. Drying under about 100 mbar (4 h, 90°C) and under HV (8 h, 100°C) results in the title compound; melting point (decomposition) 210-211°C; ESI-MS: $(M+H)^+$=466. (Yield: 70.9%)

The above reaction sequence is illustrated in the following reaction scheme:



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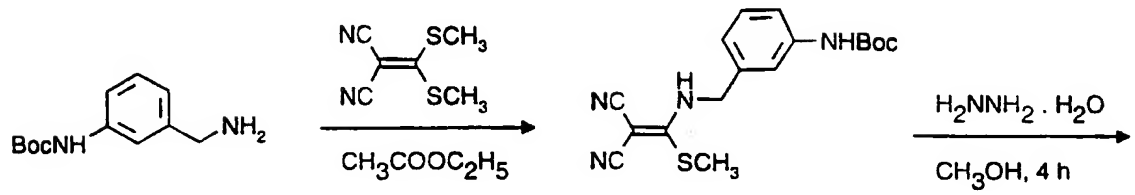
WO 93/19063



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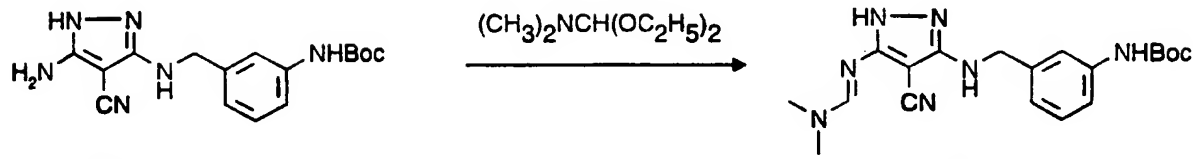

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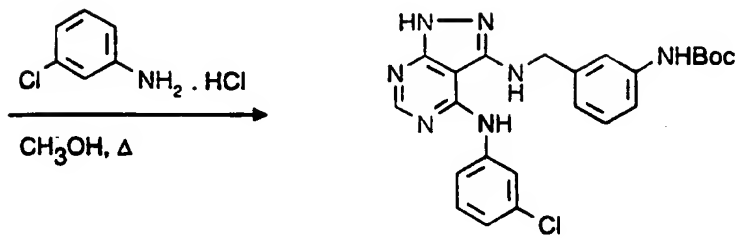

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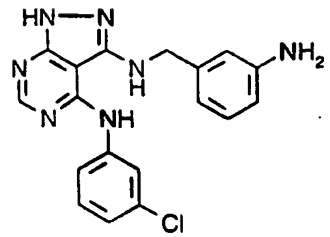


Example 3: 3-(3-Aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine mesylate

0.194 ml (3 mmol) of methanesulfonic acid is added, while stirring, to a warm solution of 1.123 g (3 mmol) of 3-(3-aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine (97.69%) in 40 ml of methanol and 20 ml of methylene chloride, and the mixture is then concentrated to about 1/3 of the original volume. The crystals which separate out on cooling to 0°C are filtered off and dried under HV (8 h, 100°C). Equilibration at 20°C under the ambient atmosphere for 24 h results in the title compound with a water content of 3.47%; melting point >120°C; ESI-MS: $(M+H)^+$=366.

Example 4: 3-(3-Aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine dihydrochloride

A mixture of 13.5 g (0.029 ml) of 3-[3-(N-Boc-amino)benzylamino]-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine and 250 ml of 3N methanolic hydrochloric acid is stirred at RT for 15 h. Then 200 ml of diethyl ether are added to the reaction mixture and, after filtration, the residue on the filter is washed with diethyl ether and dried under HV at 100°C for 8 h. Recrystallization from aqueous methanol/diethyl ether, drying under HV (12 h, 110°C) and equilibration at 20°C under an ambient atmosphere for 15 h result in the title compound with a water content of 3.94%, melting point (decomposition) 212-215°C; ESI-MS: $(M+H)^+$=366.

Example 5: 3-(3-Aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine dimesylate

A warm solution of 3.745 g (0.01 mol) of 3-(3-aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine (97.69%) in 250 ml of methanol and 250 ml of methylene chloride is concentrated to a volume of about 50 ml and, while stirring, diethyl ether is added until the mixture is slightly turbid. A crystalline precipitate forms on cooling and is filtered off and recrystallized from methanol/acetonitrile. The resulting crystals are dissolved in about 50 ml of water, the solution is filtered through Hyflow Super Cel, and the filtrate is evaporated in vacuo. Recrystallization of the residue from methanol/acetonitrile, drying under HV (8 h, 120°C) and equilibration at 20°C under an ambient atmosphere for 24 h result in the title compound with a water content of 3.58%; melting point 164-166°C; ESI-MS: $(M+H)^+$=366.

Example 6: Dry capsules

5000 capsules each of which contains as active ingredient 0.25 g of one of the compounds of the formula I mentioned in Examples 1 to 5 are produced in the following way:

Composition

Active ingredient	1250 g
Talc	180 g
Wheat starch	120 g
Magnesium stearate	80 g
Lactose	20g

Production process: The powdered substances mentioned are forced through a screen with a mesh width of 0.6 mm. 0.33 g portions of the mixture are packed into gelatin capsules by a capsule-filling machine.

Example 7: Soft capsules

5000 soft gelatin capsules each of which contains 0.05 g of one of the compounds of the formula I mentioned in Examples 1 to 5 as active ingredient are produced in the following way:

Composition

Active ingredient	250 g
PEG 400	1 l
Tween 80	1 l

Production process: The powdered active ingredient is suspended in PEG 400 (polyethylene glycol with M_r between about 380 and about 420, Fluka, Switzerland) and Tween 80 (polyoxyethylene sorbitan monolaurate, Atlas Chem. Ind., Inc., USA, supplied by Fluka, Switzerland), and ground in a wet pulverizer to a particle size of about 1 to 3 mm. 0.43 g portions of the mixture are then packed into soft gelatin capsules by a capsule-filling machine.

Example 8: In vivo antitumour activity (s.c. xenografts in nude mice) of 3-(3-amino-benzyl-amino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine (compound I)

Female Balb/c nu/nu mice (Novartis animal farm, Sisseln, Switzerland) are kept under sterile conditions (10 to 12 mice per cage Type III) with free access to food and water. Tumours are established after subcutaneous injection of cells (minimum 2 x 10^6 cells in 100 ul PBS or medium) in carrier mice (4-8 mice per cell line). The resulting tumours are serially passaged for a minimum of three consecutive transplantations prior to start of treatment. Tumour fragments (approx. 25 mg) are implanted s.c. into the left flank of animals with a 13-gauge trocar needle under Forene (Abbott, Switzerland) anaesthesia. Treatment is started when the tumour reaches a mean tumour volume of 100 mm^3.

Tumour growth is monitored twice and 24 hours after the last treatment by measuring perpendicular diameters. Tumour volumes are calculated according to the formula L x D x π /6 (Ref.: Evans et al, Brit.J.Cancer, 1982; 45: 466-468). Antitumour activity is expressed as T/C% (mean increase of tumour volumes of treated animals divided by the mean increase of tumour volumes of control animals multiplied by 100).

Treatment: Applications are given 7 days a week (p.o. or i.v.). The volumes of application are 25 ml/kg (p.o.) and 10 ml/kg (i.v.). Stock solutions of 40 mg/ml of compound I are dissolved in 100% DMSO (MERCK, Darmstadt, Germany) and stirred at room temperature until clear solutions are obtained. Prior to each administration, 10% Tween 80 (FLUKA, Buchs, Switzerland) is added to the stock solution and then diluted 1: 20 (v/v) with sterile water (p.o. applications) or NaCl 0.9% (i.v. applications). Solutions and dilutions are prepared daily prior to application.

Tumours: Human epidermoid carcinoma A 431 (ATCC: CRL 1555)

Start of treatment:	day 5 after tumour transplantation
Treatment:	once daily for 14 consecutive days
End of treatment:	day 19 after tumour transplantation
Animals:	female Balb/c nu/nu mice
	6/group
	10-14 weeks old

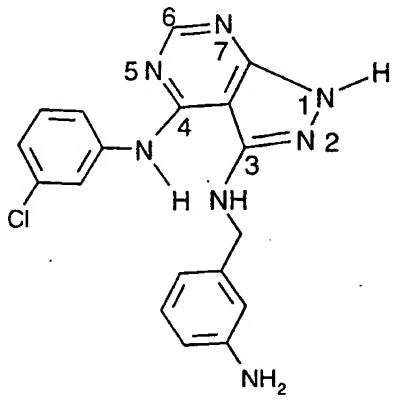
Results:

compound	dose mg/kg	no. of Appl.	T/C %	body weight **)
Vehicle	25	p.o.	100	- 1 %
Compound I	50.00	p.o.	0	+ 2 %
Compound I	10.00	p.o.	6	+ 6 %
Compound I	2.00	p.o.	7	+ 7 %
Compound I	50.00	i.v.	8	+ 7 %
Compound I	10.00	i.v.	14	+ 1 %

**) body weight changes (start of treatment versus end of treatment)

WHAT IS CLAIMED IS:

1. 3-(3-Aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine of the formula I



(I)

or a salt, solvate or tautomer thereof.

2. A pharmaceutically acceptable salt or solvate of 3-(3-aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine according to claim 1.

3. A mesylate, dimesylate or dihydrochloride salt according to claim 1.

4. A pharmaceutical composition comprising 3-(3-aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine of the formula I according to claim 1 or a pharmaceutically acceptable salt or solvate thereof together with pharmaceutical carrier material.

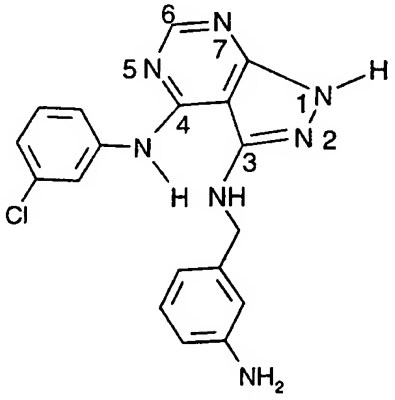
5. A pharmaceutical composition for the treatment of tumours in warm-blooded animals, including humans, comprising an effective antitumour dose of 3-(3-aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine of the formula I according to claim 1 or a pharmaceutically acceptable salt or solvate thereof together with pharmaceutical carrier material.

6. The use of 3-(3-aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine of the formula I according to claim 1 or of a pharmaceutically acceptable salt, solvate or tautomer thereof for producing pharmaceutical compositions for use for hyperproliferative disorders.

7. The use of 3-(3-aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine of the formula I according to claim 1 or of a pharmaceutically acceptable salt, solvate or tautomer thereof for the chemotherapy of tumours.

8. A method for the treatment of warm-blooded animals, including humans, wherein an effective antitumour dose of 3-(3-aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine of the formula I according to claim 1 or of a pharmaceutically acceptable salt, solvate or tautomer thereof is administered to such a warm-blooded animal suffering from an oncosis.

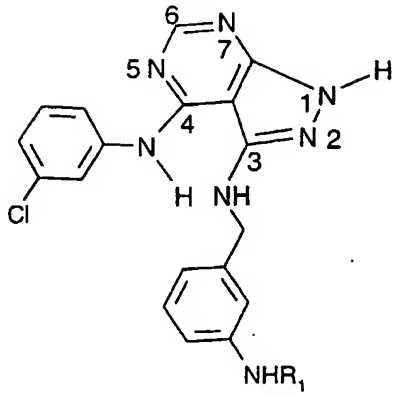
9. A process for preparing 3-(3-aminobenzylamino)-4-(3-chlorophenylamino)-1H-pyrazolo[3,4-d]pyrimidine of the formula I



(I)

or of a salt thereof, which comprises

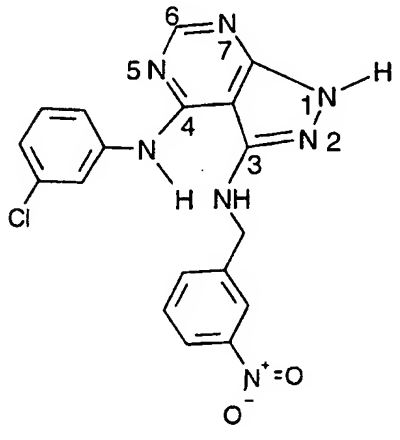
a) eliminating the protective group from a compound of the formula II



(II)

in which R_1 is an aminoprotective group, or

b) converting the nitro group in the compound of the formula III



(III)

into an amino group using a suitable reducing agent,

and if required converting a compound of the formula I obtained by process a) or b) into a salt, or converting a resulting salt of a compound of the formula I into the free compound.

INTERNATIONAL SEARCH REPORT

Interna. .ial Application No
PCT/EP 97/05377

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D487/04 A61K31/505 //(C07D487/04,239:00,231:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 19774 A (WARNER-LAMBERT) 27 July 1995 see claim 1	1,5
P,X	WO 96 31510 A (CIBA-GEIGY) 10 October 1996 see claims 1,12	1,5

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 January 1998	19. 02. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Alfaro Faus, I

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 97/05377

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 8
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 8 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat...al Application No
PCT/EP 97/05377

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